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A Summary of Current Program 3/31/69

and Preliminary Report of Progress

for 7/1/68 to 3/31/69.

PROGRESS REPORT

of the

HUMAN NUTRITION RESEARCH DIVISION

AGRICULTURAL RESEARCH SERVICE

This progress report includes a summary of the current research of the Division and a preliminary report of progress made from July 1, 1968 through March 31, 1969. It is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1968, and May 31, 1969. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Human Nutrition Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland, 20705.

UNITED STATES DEPARTMENT OF AGRICULTURE
Washington, D.C.
July 1, 1969



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INTRODUCTION

The mission of the Human Nutrition Research Division includes a broad program of basic and applied research to ascertain and make known the needs of the individual for those foods, nutrients, and diet patterns which can assure continued optimum well-being and help guide the nation's agricultural program.

The research reported here presents recent progress in understanding the nutritional needs of normal man and the manner by which these needs can best be met by food. The research involves studies of the absorption, transport, and metabolism of individual nutrients in the body as related to age, activity, heredity, and environmental conditions. Studies of metabolic processes and nutritional requirements in man are preceded, guided, and expedited by results from intensive studies on laboratory animals and lower forms of life in which more factors can be controlled and physiological responses can be measured during each stage in the life cycle and during successive generations. The research includes the nutritive values of foods as measured by chemical or physical means and by biological response, and the effects of household practices upon the nutritive value of foods. Investigations are made also of the effects of pesticide use upon human nutrition and food needs. Knowledge gained from human nutrition research can be used to influence the food habits and improve the nutritional status of man. It also can influence market demand and in turn the production of agricultural products.

The program is carried on by the Human Nutrition Research Division of the Agricultural Research Service of the U. S. Department of Agriculture. It is conducted at the Agricultural Research Center near Beltsville, Maryland, and under contract and cooperative agreement and research grants with universities, industry, and private research laboratories. In addition, the Division collaborates with Regional programs of the State Experiment Stations. The Federal Scientific effort devoted to this research in the reporting period totalled about 24.2 scientific man-years engaged in the program near Beltsville, Maryland, and at Boston, Massachusetts^{1/}. This report covers nine months rather than a full year. During this period no research contracts, grants or cooperative agreements were initiated. The program is divided among study of:

| | Intramural | Extramural | Total |
|---|------------|------------|-------|
| Human Requirements for Fats and Foods to Meet These Needs | 5.3 | 0 | 5.3 |
| Human Requirements for Minerals and Foods to Meet These Needs | 0.7 | 0 | 0.7 |
| Human Requirements for Vitamins and Foods to Meet These Needs | 0.8 | 0 | 0.8 |

| | Intramural | Extramural | Total |
|--|------------|------------|-------|
| Human Requirements for Protein and Amino Acids and Foods to Meet These Needs | 5.5 | 0 | 5.5 |
| Human Requirements for Carbohydrates and Foods to Meet These Needs | 5.5 | 0 | 5.5 |
| Effects of Pesticide Use on Human Dietary Requirements | 6.3 | 0 | 6.3 |

1/ Field location for the period June 1967 - June 1969

In previous years the research program of the Human Nutrition Research Division included intramural research on both the quality and safety of food in homes and institutions. During Fiscal Year 1969 these research activities were either discontinued or transferred to other ARS divisions. Resources for this phase of the earlier programs were redirected toward the research goals described in the present Progress Report.

Basic information on human nutrition is needed for conservation and optimal utilization of human and food resources and to promote the nutritional well-being of the population. The Division has contributed to this goal by providing information on nutritional and food needs. Some of these contributions have been summarized here:

Nutritional Status in the U.S.A. ARS human nutritionists have completed a comprehensive literature review of about 50 studies of nutritional status and 60 of diet quality made in the United States between 1957 and 1967. Increasing concern about malnutrition in the United States has highlighted the need to examine all evidence as to the extent and location of malnutrition. The studies of nutritional status and of diet quality each included information on approximately 30,000 people. Results were compiled with respect to age for height and weight, protein nutrition, incidence of anemia, vitamin status, and dietary evaluation. Poor nutritional status was found among the low-income families, particularly infants, children, and older people. Anemia was common in pregnant women and preschool children. Quality of nutrition and diet was generally related to economic status and level of education of the heads of the households. The nutrients most often in short supply in the diets were calcium, iron, and vitamins A and C.

Magnesium Requirements of Adolescent Boys Determined. Recent studies indicate that magnesium requirements of humans are appreciably higher than have been previously estimated. ARS sponsored research at the University of Wisconsin showed that 17-19 year-old boys on a controlled

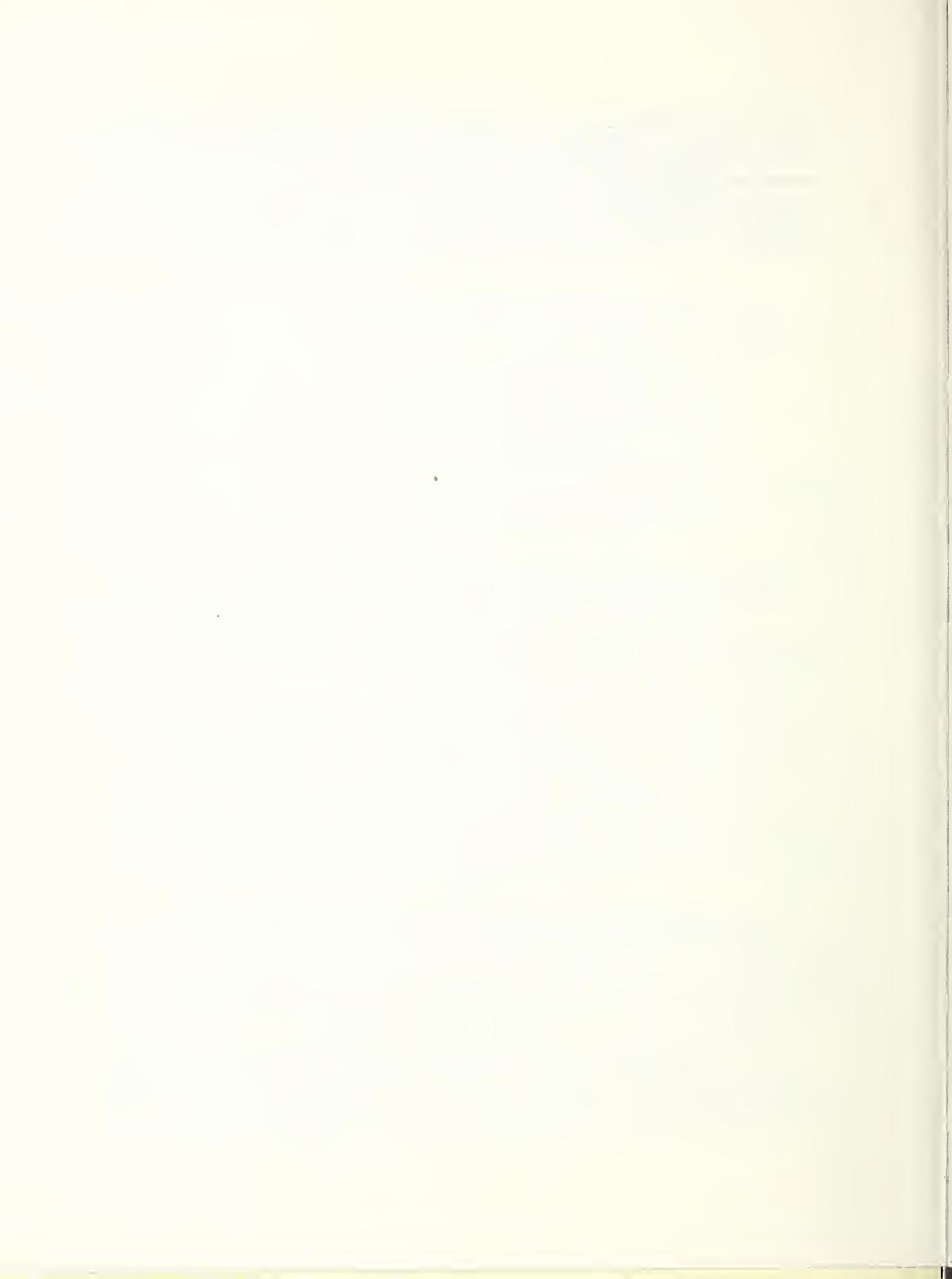
diet needed approximately 580 mg of magnesium per day which is 45 percent more than the NAS-NRC Recommended Allowance of 400 mg per day. Earlier ARS sponsored research also indicated higher magnesium requirements than the recommended allowance for men aged 21-28 years. Magnesium plays an essential role in the body's use of food. The higher requirements are significant in view of the constantly decreasing level of magnesium per capita in the national food supply.

Collagen May Now Be Studied In Natural State. Previously inaccessible intramuscular connective tissue from striated and cardiac muscle and containing the protein, collagen, has been isolated in high purity and without physical or chemical alteration. The new procedure, developed by the Human Nutrition Research Division biochemists, for the first time makes it possible to study these important framework tissues of muscle in their natural state. These muscle components are closely associated with the membranes through which nutrients enter and leave muscle cells. Changes in collagen associated with growth, old age, vitamin E deficiency, and nutritional status may now be identified and studies made of the effects of diet in preventing adverse changes. The new procedure already is finding wide use by scientists engaged in medical, biochemical, and food research.

New Vitamin E Forms Found In Corn, Coconut, Barley And Other Seeds And Oils. ARS human nutritionists recently have found a number of forms of vitamin E in seeds and oils which had not previously been known as sources of these compounds. One of these forms of vitamin E previously had been reported and properly identified only in palm oil, latex, and human bone marrow. We have now found it to be a major vitamin E form in both fresh corn and coconut oil, and to occur in smaller amounts in barley. The vitamin E potency of this compound is not known, but in all three sources examined, its concentration equalled or exceeded that of alpha-tocopherol, the vitamin E standard. The studies also have shown that the vitamin E components of coconut oil, commonly used in filled milks, may contribute only three milligrams per 100 grams of oil. Soybean oil may contain 80 milligrams of the tocopherols per 100 grams. Dietary allowances for vitamin E were first recommended in 1968 by the National Research Council.

High-Protein Oilseed Flours Satisfactorily Used In Home Breadmaking Procedures. Scientists at the Human Nutrition Research Division have succeeded in producing high-quality breads and chapatis (an unleavened bread typical of India) when as much as 30 to 40 % of high-protein oilseed flours are substituted for wheat flour. Current breadmaking technology permits the incorporation of only 5 to 10% of these oilseed flours before quality is seriously affected. Based on fundamental studies of dough properties, the scientists have modified bread formulations and breadmaking procedures to permit the incorporation of higher levels of high-protein soy and cottonseed flour into breads of various types, thus substantially raising the protein content. The rapidly increasing world population

is forcing consideration of alternate protein food sources. Wheat, corn, and rice flours used in breadmaking are low in protein as compared to the oilseed flours. Oilseeds represent an enormous and virtually untapped source of food protein. The problem is to prepare the high protein flours from oilseeds in an acceptable and appetizing form. Incorporation of oilseed flours such as soy and cottonseed into breads is an excellent way of increasing protein consumption.



AREA NO. 1: HUMAN REQUIREMENTS FOR FATS AND FOODS TO MEET THESE NEEDS

(RPA 708 - HUMAN NUTRITIONAL WELL-BEING)

| Location of Intramural Work | Scientist Man-years FY 1969 | | |
|---|--------------------------------|------------|-------|
| | Intramural | Extramural | Total |
| Maryland (Beltsville) | 5.3 | 0 | 5.3 |
| Domestic program is supplemented by PL 480 funds in Israel and India. | | | |

Problems and Objectives

A major health concern is with determining the optimal amount of fat needed in the diet and perhaps of even greater importance, the type of fat. There is ample evidence that amount and type of fat are important, but present knowledge is insufficient to arrive at general recommendations for dietary fat. Over the years there have been changes in both the amount and type of fat consumed in U.S. diets. Also, technology advances have brought about changes in fats. The changes have nutritional significance but are inadequately documented.

Nutritional requirements must be expressed in terms of foods and diets if advances in nutrition knowledge are to benefit people. The relationship of dietary fat to heart disease, atherosclerosis, blood coagulation and thrombosis has been suggested by several studies but the cause and effect hypothesis has not been proven. When nutrition research has progressed sufficiently that specific dietary recommendations can be made, it may be possible to modify a sizable percent of the heart and vascular cases and increase the productive lifespan and work efficiency of people. The magnitude of the potential benefits can be appreciated if one notes that some 28 million adults in 1960-1962 were diagnosed or suspected to have heart and vascular disease. The economic costs of death from heart disease were calculated at nearly \$32 billion annually.

Major objectives of the research are to develop recommendations for fat intake by humans and to identify the amount, kind, and assortment of foods needed. This will involve:

1. Identifying the individuals or groups of individuals who could benefit from dietary regulation of fat and the age the regulation should begin.

2. Finding out what fat constituents, such as cholesterol and fatty acids in the diet, need to be regulated and how they should be regulated.
3. Determining the amount, composition, and availability of the various lipid components in food.
4. Determining the extent and manner by which non-fat diet constituents may influence recommendations for fat intake.

Progress - USDA and Cooperative Program

A. Fatty Acids of Food Products

A study of the fatty acids of wheat and wheat products which was part of a larger, multi-nutrient study has been completed. Samples of soft white, hard red, and durum wheat were obtained from commercial graneries in principal growing areas. These were milled into flour and processed wheat products were made from these flours. Ten samples each of a variety of wheat products available to the consumer were obtained from markets in ten cities in the United States. These products included flour, breads, rolls, and cakes. Fatty acid analysis of this variety of wheat products indicated that there was a marked uniformity in the fatty acid makeup regardless of geographical location. The lipids of wheat grains and of their corresponding flours, although amounting to only 2.5 to 3.2%, were largely linoleate acid, this amounting to over half of the total fatty acids. These lipids also contained linolenic acid, a more highly unsaturated fatty acid.

A study of the fatty acids and fat fractions of different raw and cooked beef cuts indicates that fatty acid composition is not changed by either moist or dry cooking, although the absolute amounts of the fatty acids decrease through drip losses. The changes during maturation were followed by analyzing biopsy samples taken from the longissimus dorsi muscle at two-month intervals. Fatty acid composition of muscle lipid from biopsy samples varied with animal age, total amount of lipid, and season. Saturated fatty acids increased and oleic acid decreased between the months of March and May. Arachidonic acid varied with season, being lower in the cold months of November and January than in other months. The influence of season was in general greater than that of age. It was observed that the stearic acid of muscle phospholipids decreased with increasing age. Postmortem examination of the carcasses revealed that many of the biopsy sites had been infiltrated with fat and contained about 65% lipid; normal tissue contained about 16%. The fatty acid composition of the degenerated muscle was similar to that of subcutaneous fat depots. The mechanism of this change is not known, but may have been caused by accidental denervation during biopsy.

B. Cholesterol Metabolism

A long term study has focused on the effects of heredity, diet, sex, and body and organ size on serum cholesterol and other aspects of lipid

metabolism. To study individual differences in lipid metabolism, inbred lines of the BHE rat were developed with differing obesity. Various body measurements were found to be useful predictors of body fat if the age, sex, and strain of the animal are known. Weight by itself is not a reliable indicator.

Further information has been obtained through PL 480 research in India on the role of estrogen in controlling cholesterol metabolism. The administration of 17 β estradiol to rats inhibited the formation by the liver of hydroxymethyl-glutaryl-CoA, a precursor of cholesterol. A specific inhibition of the enzyme forming this compound was proposed as the means through which the hormone influences accumulation of cholesterol. This research group also is reporting on the effects of dietary myristic acid on the cholesterol metabolism of the rat.

C. Dietary Fat and Serum Proteins

The serum proteins of rats fed diets varying in type and level of fat were studied with high resolution disc electrophoresis. The level of prealbumin, a protein associated with lipid in the serum, was affected by the level of fat in the diet. The level of transferrin-2, another serum protein, varied with the type of fat.

Publications - USDA and Cooperative Program

Lakshmanan, F. L., and Schuster, E. 1969. Changes with age in levels of serum proteins in rats fed diets differing in amount and kind of fat and carbohydrate. Fed. Proc. 28:305 (Abstract).

Link, B. A., Bray, R. W., Cassens, R. G., and Kauffman, R. G. 1968. Bovine muscle protein solubility during growth. J. Anim. Sci. 27:1143 (Abstract).

Link, B. A., Cassens, R. G., Bray, R. W., and Kowalczyk, T. 1967. Fatty degeneration in bovine longissimus. J. Anim. Sci. 26:654.

Mukherjee, A., and Bhose, A. 1968. Studies on estrogen regulation of cholesterol biosynthesis in rat liver microsomes. Biochem. Biophys. Acta 164:357.

AREA NO. 2: HUMAN REQUIREMENTS FOR MINERALS AND FOODS
TO MEET THESE NEEDS

(RPA 708 - HUMAN NUTRITIONAL WELL-BEING)

| Location of Intramural Work | Scientist Man-Years FY 1969 | | |
|-----------------------------|--------------------------------|------------|-------|
| | Intramural | Extramural | Total |
| Maryland (Beltsville) | 0.7 | 0 | 0.7 |

Domestic program is supplemented by PL 480 funds in India, Japan, and Poland.

Problems and Objectives

Although the human body contains a large variety of mineral elements, Recommended Dietary Allowances issued by the National Academy of Sciences-National Research Council have been proposed for only five minerals. Prior to 1968, only calcium and iron were included. This reflects the dearth of evidence for both minimum and maximum intake of a variety of essential mineral elements known to be required by man. Even when these requirements are known, they must be translated into terms of food and diets if advances in nutritional knowledge are to benefit people. Benefits from mineral nutrition research may be expected to include improved health, a longer productive life, and fewer work days lost. The research leads suggest that optimal intake of minerals in the diet could lead to stronger and better developed bones and teeth with less likelihood of deterioration as in osteoporosis and tooth decay, a lower incidence of anemia with associated reduced stamina and activity, reduced incidence of goiter, a lowering of the incidence of cardiovascular disorders, and numerous other health benefits that can be translated into economic benefits.

Major objectives of the research are aimed at developing recommendations for mineral intake by humans and identification of the amount, kind, and assortment of foods needed. This includes research to:

1. Identify the individuals or groups of individuals who could benefit by altering or regulating their dietary mineral intake.
2. Identify the minerals which need to be regulated in the diet and how they should be regulated.

3. Determine the amount, composition, and availability of the mineral components of food.

Progress - USDA and Cooperative Program

A. Minerals in Wheat Products

A study has been made of the mineral contribution of a variety of wheats and the flours and various baked products made from them, as well as of ten different consumer products collected at each of ten different geographical locations in the U. S. This was part of a larger multi-nutrient analytical program now nearing conclusion. Data for total ash, iron, phosphorus, potassium, sodium, calcium, and magnesium were not greatly different among the products available to the consumer in different areas in the U. S. There was almost twice as much iron in semolina as in bread or cake flour, although durum wheat was not greatly different in iron or other elements when compared with the hard and soft wheat samples. In recent years many commercial bakeries have changed their bread making procedures and in so doing, have often changed their dough formulations. This did not appear to noticeably change the nutritive value of the bread; calcium values for breads prepared from continuous mix and the more conventional intermittent mix dough preparations were the same.

B. Magnesium and Calcium Requirements

The results of a study with 17-19 year old boys conducted under contract at the University Wisconsin have shown that the boys' requirement for magnesium under the conditions of this study was about 580 mg per day. This is 45% higher than the currently Recommended Dietary Allowance of 400 mg per day. The data collected during the 60-day study showed also that the level of protein in the diet affected the absorption and urinary excretion of magnesium and calcium. Two levels of protein were fed (approximately 45 and 135 g per day). Mineral absorption was greater with the higher protein intake. The amount of calcium excreted in the urine with the high protein diet was greater than the amount of calcium ingested. A concomitant increase in the urinary excretion of glucosamine suggested that calcium was being withdrawn from the bone. If this proves to be true, it may suggest need for changes in dietary practices currently recommended to increase calcium intake or to treat such conditions as osteoporosis.

Kinetic studies of calcium metabolism with rats fed either 0.5 or 1.5% calcium in the diet showed that calcium pool size, rate of intestinal absorption, and bone calcium deposition reached a maximum at seven weeks of age and had not changed further at 16 and 32 weeks. The diet had little effect on rate of bone deposition and no effect on serum calcium. Pool size and rate of absorption were influenced by dietary calcium level. Rate of absorption seemed independent of rate of bone deposition. This investigation is being carried out under a research grant at the University of Louisville.

Publications - USDA and Cooperative Program

Alcantara, E. N., and Linkswiler, H. 1969. Effect of protein and magnesium intake on magnesium balance in older adolescent males. Fed. Proc. 28: 562. (Abstract).

Bronner, F. 1969. Calcium Homeostasis. 156th American Chemical Society National Meeting, Atlantic City. (Abstract).

Bronner, F., Stacey, R. E. and Change, S. I. 1969. Calcium metabolism parameters as a function of age and calcium intake. Fed. Proc. 28: 591. (Abstract).

Edwards, C. H., Booker, L., Craven, R., and Ganapathy, S. N. 1969. Utilization of calcium, magnesium, phosphorus and sulfur in wheat by adult man. Fed. Proc. 28: 562. (Abstract).

Hepburn, F. N., and Tulloss, J. H. 1969. Mineral elements in wheat and wheat products: Content of ash, iron, phosphorus, calcium, magnesium, potassium, and sodium. Cereal Sci. Today 14 (Abstract).

Johnson, N. E., and Linkswiler, H. 1969. Effect of level of protein and magnesium intakes on calcium retention in older adolescent males. Fed. Proc. 28: 562. (Abstract).

AREA NO. 3: HUMAN REQUIREMENTS FOR VITAMINS AND FOODS TO MEET THESE NEEDS

(RPA 708 - HUMAN NUTRITIONAL WELL-BEING)

| Location of Intramural Work | Scientist Man-Years FY 1969 | | |
|-----------------------------|--------------------------------|------------|-------|
| | Intramural | Extramural | Total |
| Maryland (Beltsville) | 0.8 | 0 | 0.8 |

Domestic program supplemented by PL 480 funds in India, Israel, Japan, and Poland.

Problems and Objectives

Recommended dietary allowances for ten vitamins essential to man have been proposed by the National Academy of Sciences - National Research Council. Four of these (folacin and vitamins B₆, B₁₂, and E) were included for the first time in the last revision in 1968. Dietary allowances recommended are for some vitamins based on incomplete information for a number of the age and sex groups. A number of other vitamins are known to be required but information is too fragmentary to provide a basis for establishing recommendations. Recent information from both dietary surveys and nutritional status studies indicates that inadequate dietary intakes of vitamins may be found at all income levels in the U. S.

Human requirements for vitamins must be expressed in terms of foods and diets if advances in nutritional knowledge are to benefit people. Although the major vitamin deficiency diseases in the U. S. have been reduced to a very low level, less obvious but debilitating aspects of improper nutrition are widespread. For example, folacin deficiency is known to contribute to the widespread anemia being observed in nutritional status surveys. Benefits from nutrition research in this area include improved health, a longer productive life, a greater sense of well-being, and fewer work days lost. Major economic benefits would derive from these gains.

Major objectives of the research focus on the goal of developing recommendations for vitamin intake by humans and identification of the amount, kind, and assortment of foods needed to supply the vitamin needs. This includes the following research aims:

1. Identify the individuals or groups of individuals who could benefit from altering or regulating their dietary vitamin intake.
2. Identify the vitamins which need to be regulated in the diet and how they should be regulated.
3. Determine the amount, composition, and availability of the vitamin components of food.

Progress - USDA and Cooperative Program

A. Vitamins in Wheats and Wheat Products

Intramural research and studies being carried out under a research contract with the American Institute of Baking are providing further information on nutrients in wheat products. These studies included a number of types of wheat, flours milled from these samples, and baked products prepared from the flours. The studies also included a variety of consumer products in markets in ten regions in the U. S. Little of the tocopherol (vitamin E) content of the wheat grain was found to be retained in the flour. Both the type and quantity of tocopherols in consumer products reflected the type and quantity of shortening added. There were regional differences in baked products in the consumer markets, principally in the non-wheat gamma and delta tocopherols. Alpha tocopherol, the usual standard for vitamin E, was not the major tocopherol form in any product.

Pyridoxine generally accounted for about three-fourths of the total vitamin B₆ forms in wheats. Durham wheat contained more vitamin B₆ than hard or soft wheats. In milling, the semolina from durum wheat retained some 28% of the vitamin, whereas hard wheat flour retained only 15% of the vitamin in whole wheat. In the milling of soft wheat, the cake flour contained only 10% of the original vitamin B₆, and the cracker flour from regular milling straight grade or air classified milling contained only slightly more. The cut-off fraction from soft wheat milling, also used for crackers, contained as much as 25% of the original vitamin B₆. Less than 15% of the vitamin B₆ of whole wheat was found in bread, 7% in cake, 10 to 20% in crackers, and 25% in macaroni. The small percentages of the vitamin retained in baked products reflected the minor amounts retained during milling rather than losses during baking. Differences in vitamin B₆ in consumer products were not related to market location.

The niacin in cereal products has a significant fraction occurring as a bound form believed to be nutritionally unavailable. Generally, half of the niacin of wheats and wheat products was found to be in the free form. Exceptions occurred in products where niacin was added to the recipe by ingredients other than flour. Again, the consumer products such as flour, cereals, bread, and rolls were quite uniform in niacin content throughout the ten areas in the U. S. sampled. Durum wheat had substantially more niacin than the hard and soft wheats, and milled products from durum retained more of this niacin than during the milling of hard and soft wheats.

B. Nutrients in Soybean Products

Tempeh is a fermented soybean product that is a dietary staple in Indonesia and other areas. Data from PL 480 research in Japan indicate that the nutritive value of tempeh is greater than that of unfermented soybeans. Repeated studies with rats showed tempeh to be superior in hemolysis tests and in thiobarbituric acid values of the livers. This was accompanied by higher serum tocopherol levels. These relate to a factor in tempeh produced by the mold fermentation. This factor has antioxidant properties and has been identified as 6,7,4'-trihydroxyisoflavone. Further purification of the enzyme responsible for the liberation of this factor has been reported.

C. Vitamin A Utilization

PL 480 research in Poland has led to development of a standard procedure for measuring carotene or vitamin A availability from food. The method depends upon the use of a liver test with rats containing a low initial vitamin A reserve in the liver. Optimal time and intake levels were investigated. These were standardized at a ten day feeding period with 30 micrograms of beta-carotene or 30 IU of vitamin A per rat per day. Five subcellular protein fractions were obtained from the liver and the vitamin A distribution in these fractions was determined. The results obtained suggest that the mitochondrial and nuclear fractions are probably responsible for vitamin A storage in the liver.

D. Availability of Folacin from Foods

Folate deficiency is frequently found among pregnant women, children, and the elderly in spite of a food supply which is believed to contain adequate amounts of folates. A project to be conducted in Israel under a PL 480 grant has been initiated to study this paradox. In this project, the biological activity of folate-containing foods subjected to various customary methods of cooking will be studied with rats. Using radioactive folate, the absorption of folate from various food combinations will be estimated in normal and folate-deficient rats. Corroboration of the results obtained with rats will be sought in studies with human subjects, including normal healthy persons and others from population groups susceptible to nutritional anemia.

Publications - USDA and Cooperative Programs

Gronowska-Senger, A., Roszkowski, W., Daniewski, M., and Berger, S. 1968. Relationship between the level of vitamin A and its distribution among some protein fraction of rats liver. Sci. Rep. Warsaw Inst. Agr. Technol. Agr. and Food, 35:67. (Polish with English summary).

Hepburn, F. N. 1969. "Free" and "bound" niacin in wheat and wheat products. Cereal Sci. Today 14 (Abstract).

Ikehata, H., Wakaizumi, M., and Murata, K. 1968. Antioxidant and anti-hemolytic activity of a new isoflavone, "Factor 2", isolated from tempeh. Agr. and Bio. Chem. 32:740.

Korycka, M., Daniewski, M., and Berger, S. 1968. Standard procedure for the evaluation of the utilization of the compounds showing the vitamin A activity in the body. Sci. Rep. Warsaw Inst. Agr. Technol. Agr. and Food, 35:89. (Polish with English summary).

AREA NO. 4: HUMAN REQUIREMENTS FOR PROTEIN AND AMINO ACIDS
AND FOODS TO MEET THESE NEEDS

(RPA 708 - HUMAN NUTRITIONAL WELL-BEING)

| Location of Intramural Work | Scientist Man-Years FY 1969 | | |
|----------------------------------|--------------------------------|------------|-------------------|
| | Intramural | Extramural | Total |
| Maryland (Beltsville) | 4.7 | 0 | 4.7 |
| Massachusetts (Boston) <u>1/</u> | 0.8 | 0 | <u>0.8</u> 5.5 |

Domestic program supplemented by PL 480 funds in India and Egypt.

Problems and Objectives

Proteins and amino acids are the key components in tissue growth and development. There is increasing evidence in the scientific literature that a deficiency of these constituents during certain critical periods in human development can seriously and perhaps irreparably impair not only physical development but mental development as well. Protein deficiency constitutes one of the major deficiency diseases throughout the world. Nevertheless, knowledge of human requirements for protein and amino acids has many gaps. The defining of requirements is made difficult by the complexities of interrelationships with other nutrients and among the amino acids themselves. These nutritional requirements must be expressed in terms of foods and diets before advances in nutritional knowledge can benefit people. Diet planning thus is dependent upon knowledge of the kind, amount and biological availability of proteins and their constituent amino acids in our food supply.

Major objectives of the research are to:

1. Identify the individuals or groups of individuals who could benefit from altering or regulating their dietary intake of protein and amino acids.

1/ Field location for the period June 1967 - June 1969.

2. Identify the protein constituents which need to be regulated in the diet and how they should be regulated.
3. Determine the amount, composition, and biological availability of the protein and amino acid components of foods.

Progress - USDA and Cooperative Program

A. Human Requirements

As part of a comprehensive review of current knowledge of human requirements for nutrients, a literature search of past research on requirements for proteins and amino acids has been completed. This phase of the study was carried out in cooperation with the Harvard University School of Public Health. Despite a voluminous literature, the review reveals that many of our currently held beliefs on protein and amino acid requirements for different population groups are based on studies of very few individuals using techniques of questionable reliability. A manuscript is being prepared for publication.

Studies are underway at the Massachusetts Institute of Technology under a grant to assess the usefulness of the plasma amino acid response curve to measure individual essential amino acid requirements of adults. Preliminary tests have been completed with seven young male adults fed decreasing amounts of lysine, and four fed decreasing amounts of tryptophan. Further study of the method is in progress.

B. Utilization of Non-Essential Nitrogen

Preliminary findings of a difference between the utilization of α -amino and amide nitrogen were not substantiated in further nitrogen balance studies with aging rats. Findings have been accepted for publication in the Journal of Food Science.

It was found that young male rats fed equal, restricted amounts of diets containing low but presumably adequate levels of essential L-amino acids made equally efficient use of the nitrogen of glutamic acid, aspartic acid, or a mixture of four other nonessential amino acids for nitrogen gains. The nitrogen of diammonium citrate was only 85% as well utilized as that of the other compounds. At the same restricted calorie intake, nitrogen was more efficiently used when water was added to a diet containing low levels of essential amino acid nitrogen and glutamic acid nitrogen. The response to added water was abolished when the amount of glutamic acid nitrogen was doubled. The reason for the effect of water added to the diets is unknown. A manuscript incorporating these data will appear in the Proceedings of the Society for Experimental Biology and Medicine.

C. Adverse Effects of Improper Dietary Protein

The importance of adequate quantity or quality of protein in the diets of both the mother and offspring is reinforced by research under a PL 480

grant at the University of Baroda, India. Diets low in protein or containing protein of poor quality impaired psychological performance of young rats and lowered amino acid metabolizing enzymes in the brain. Mere caloric restriction had no such effect.

D. Nutritional Availability of Fructose-Amino Acid Compounds

The compound formed from heating methionine with glucose and referred to in last year's Report has been identified as l-deoxy-l-methionino-D-fructose. This compound proved to be unavailable to rats as a source of dietary methionine. A fructose-glycine compound has now been prepared, characterized, and tested as a source of non-essential nitrogen for the rat. Findings are being prepared for publication.

E. Characterization of Muscle Proteins

The fibrillar proteins actin, myosin, and tropomyosin B and the collagenous protein tropocollagen from intramuscular connective tissue have been isolated from bovine and porcine muscle, purified, and partially characterized by physical and chemical techniques. Tropomyosins from ovine and civet muscle have been similarly isolated and studied. Marked variations in the sub-unit composition of salt-and acid-soluble tropocollagens have been observed, indicating apparent species differences in inter- and intramolecular cross-linking. Cross-linking and/or synthesis characteristics also differed according to muscle source, marked dissimilarities being observed between tropocollagen isolated from the intramuscular connective tissue of the l. dorsi and semimembranosus muscles. Ultracentrifugation, viscosity, and amino acid composition data have been accumulated on the purified fibrillar proteins.

The findings in this work will be of eventual benefit in elucidating the relation of collagen cross-linking to aging, the general physiological response of connective tissue to diet, and the influence of degree of cross-linking upon quality characteristics of meat as a food. Likewise, the observations on fibrillar proteins will be useful in assessing the nutritional importance of the structural and chemical properties of these proteins with particular reference to the subsequent nutritional availability of their constituent amino acids.

F. Protein Supplementation of Bread

One means of improving protein nutrition is to increase the protein content of some of the foods common in diets. Oilseed flours offer an attractive opportunity of raising the level of dietary protein if they can be incorporated into acceptable food formulations. Effects of adding oilseed flours such as cottonseed, peanut, safflower seed, and soy on doughs and breads were investigated. The farinograph, extensigraph, amylograph, and various histological staining techniques were used as tools for measuring performance of oilseed flours. Results of these measures were used in developing principles for the use of oilseed flours in doughs.

Oilseed flours increased absorption and usually decreased mixing tolerance of doughs concomitantly with increases in level of replacement of wheat flour. Some soy flours increased mixing tolerance of doughs. Oilseed supplemented breads made by the straight-dough method were usually poor in volume. Adjustments in the formulation or the mixing time of doughs, or both, usually improved the loaf volume of breads.

Publications - USDA and Cooperative Program

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AREA NO. 5: HUMAN REQUIREMENTS FOR CARBOHYDRATES AND
FOODS TO MEET THESE NEEDS

(RPA 708 - HUMAN NUTRITIONAL WELL-BEING)

| Location of Intramural Work | Scientist Man-years FY 1969 | | |
|-----------------------------|--------------------------------|------------|-------|
| | Intramural | Extramural | Total |
| Maryland (Beltsville) | 5.5 | 0 | 5.5 |

Domestic program supplemented by PL 480 funds in Israel.

Problems and Objectives

In recent years nutritionists have moved away from the concept of the carbohydrates being essentially calorie-providers, with the different types of carbohydrates being similar in most nutritional respects. This narrow viewpoint failed to indicate any urgency for studies on the various carbohydrate components of foods. The result is that much of our information on carbohydrate content of commodities is a residual figure of carbohydrate "by difference." It is now known that many species and many strains of animals as well as individual human beings show quite different metabolic responses to different dietary carbohydrates. These studies suggest that some of the benefits of carbohydrate nutrition research may relate to heart and vasculatory diseases, which were diagnosed or suspected in 28 million adults during 1960-62. The economic costs of death from heart disease have been calculated at approximately \$32 billion annually. When nutrition research has progressed sufficiently that specific dietary recommendations can be made, it probably will be possible to modify a portion of the heart and vasculatory cases and increase the productive lifespan and work efficiency of people who may differ in their metabolism of dietary carbohydrates.

Major objectives of the research are to:

1. Identify the individuals or groups of individuals who could benefit from altering or regulating their intake of dietary carbohydrate.

2. Identify the carbohydrates which need to be regulated in the diet and how they should be regulated.
3. Determine the amount, composition, and biological availability of the carbohydrate components of food.

Progress - USDA and Cooperative Program

A. Carbohydrates of Wheat and Wheat Products

In a multi-nutrient analytical study, a number of samples of wheats and of flours and products made from them were analyzed for a variety of carbohydrates. In addition, 10 different types of wheat products in consumer markets were collected at each of ten different geographical locations in the U.S. and their carbohydrates were determined. Among the carbohydrate components measured were: reducing and non-reducing sugars, starch, lactose, pentosans, and crude fiber. Similar consumer products from different geographical areas did not differ significantly in the carbohydrate constituents. In these foods, measurable levels of pentosans, and crude fiber components were detected only in the whole wheat products. Milling of wheat to flour resulted in a decrease in reducing and non-reducing sugars and an increase in starch content of all of the flours. Carbohydrate components of the product prepared from these flours reflected the additions of non-flour carbohydrates. A manuscript incorporating these data will appear in Cereal Chemistry.

B. Nutritional Significance of Individual Carbohydrates

A study to determine biological influences of long-term feeding of particular kinds of carbohydrate is being carried out under a research contract at Falls Church, Virginia. In this study, strains of rats have been used as models of individual genetic differences which may influence nutritional response. Diets differed only in the kind of carbohydrate provided. The carbohydrate was found to influence growth, size of organs, and cholesterol levels in liver and serum. These diet effects differed markedly among the strains of animals. Dietary carbohydrate influenced survival in only one of the three strains studied. Charles River animals which were fed the sucrose diet showed increased mortality over those receiving the starch diet. It was further observed that Charles River animals which were fed the sucrose diet up to 150 days of age and then switched to the starch diet came to resemble in most respects animals fed the starch diet from the start. However, survival was not improved by this dietary change. These findings parallel earlier results of intramural research on still additional strains of rats. They suggest that nutritional involvements of individual carbohydrates are important, though complex and variable; carbohydrate nutrition early in life may be important in determining ultimate lifespan.

Research has been initiated to determine metabolic processes that are influenced by dietary sucrose. This work is being carried out in rats under a grant at the Hebrew University-Hadassah Medical School in Jerusalem and focuses on metabolic processes related to the synthesis of lipids and protein, particularly in vascular tissues.

C. Carbohydrate and Nitrogen Interrelationships

In *T. pyriformis*, a protozoan whose amino acid requirements closely parallel those of higher animals, the type of carbohydrate in the growth medium has a pronounced effect on the pattern of free amino acids that are accumulated by cell pools and from which the cell must draw for protein synthesis. While it has been shown earlier that this organism grows better when carbohydrate is supplied as a polysaccharide rather than as a simple sugar, recently completed experiments indicate that this effect of type of carbohydrate on growth is independent of its effect on the composition of amino acid pools. When *T. pyriformis* was grown in media with amino acid assortments simulating those of cell pools from protozoa grown in media with dextrin or with glucose, growth in both media was more rapid and achieved a greater maximum with carbohydrate supplied as a polysaccharide. The pattern of bound amino acids, however, was found to be independent of alterations in patterns of free amino acids that were associated with type of media carbohydrate.

Publications - USDA and Cooperative Program

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Chang, M. L. W., Schuster, E. M., Lee, J. A., Snodgrass, C., and Benton, D. A. 1968. Effect of diet, dietary regimens and strain differences on some enzyme activities in rat tissues. J. Nutrition 96: 368.

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Reynolds, H. 1969. An Apparent Carbohydrate-Amino Acid Interaction in *Tetrahymena pyriformis*. J. Protozool. 16: 204.

AREA NO. 6: EFFECTS OF PESTICIDE USE UPON HUMAN NUTRITION

(RPA 701 - INSURE FOOD PRODUCTS FREE OF TOXIC RESIDUES FROM AGRICULTURAL SOURCES)

| Location of Intramural Work | Scientist Man-Years FY 1969 | | |
|-----------------------------|--------------------------------|------------|-------|
| | Intramural | Extramural | Total |
| Maryland (Beltsville) | 6.3 | 0 | 6.3 |

Problems and Objectives

Pesticide residues are found in almost every food eaten by the consumer. The residue levels in human diets are below those allowed by Federal regulation. However, very little is known about the effects of intake of low levels of pesticides over long periods of time upon the metabolism of nutrients in humans. Research has concentrated on the toxic effects of the pesticide chemicals. There are also indications that the nutrient content and value of foods may be altered by the presence of pesticides during growth. As agricultural management practices change, it may be necessary to revise some of the figures in food composition tables insofar as pesticide usage may alter these nutritional values. Findings from research in this area can guide the selection of pesticides for use in food production. The agriculture economy benefits by increased production efficiency. Individuals benefit from improved nutrition and its contribution to improved health, a longer productive life, a greater sense of well-being, and more satisfaction with the foods they eat.

Major objectives of the research are to:

1. Determine the effects of long-term, low level intakes of pesticides upon nutrient requirements of people.
2. Determine whether proper diet may protect against adverse effects of environmental levels of pesticides in the diet.
3. Identify and measure the effects of the use of pesticides during production upon the nutritive value of foods.
4. Determine whether and how diet and food use recommendations need to be adjusted as the result of the use of pesticides under allowed procedures.

Progress - USDA and Cooperative Program

A. Thyroid Growth and Insecticides

Evidence has been obtained indicating that iodine requirements may be significantly influenced under conditions of dietary stress such as may occur when diets contain high levels of some of the chlorinated hydrocarbon insecticides. In feeding studies with rats, thyroids showed signs of iodine deficiency that were significantly more pronounced in those rats fed lards containing high levels of DDT or heptachlor than in the control animals or in those containing levels of DDT or heptachlor within the range currently considered acceptable. The diets were marginal with respect to iodine needs. Hearts, aortas, lungs, livers, adrenals, kidneys, stomachs, testes, thyroids, pituitaries, and the brains were weighed and examined histologically. There were no differences among the groups except for the thyroids. The lards from hogs fed or sprayed with malathion contained insignificant amounts of malathion and when fed to rats the thyroids were comparable to those in the control animals.

B. Pesticide-Zinc Interrelationships

Research to determine the effects of chronic low levels of insecticide intake of rats under stresses of pregnancy when level of zinc in the diet is varied is providing little evidence of any significant influence of insecticides on physiological response. However, some differences due to level of zinc were observed. The levels of zinc studied were 3 ppm, 50 ppm, and 7,000 ppm. The insecticide mixture contained DDT, parathion, and carbaryl. All rats fed the high zinc diet and their fetuses showed a significant reduction in hemoglobin levels. Weight gains and fetal weights were lower with the high level of zinc. Red blood cell uptake of ⁶⁵Zn administered to the mothers just prior to birth of their young was highest in the rats fed the low level of zinc, probably an attempt to replenish depleted zinc stores. Level of zinc also influenced significantly the liver DDT content. These studies were carried out under contract research at the University of Florida.

Publications - USDA and Cooperative Program

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